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Appln. Serial No.: 09/882,509 ^{75 710 - 6} Fil. D. 27 Group Art Unit: 1652
Filing Date: June 15, 2001 Examiner: Monshipouri, Maryam
Applicant(s): Kuppusamy et al. ^{US PATENT & TRADEMARK OFFICE} Attorney Docket No.: 51321:003 ^{US PATENT & TRADEMARK OFFICE}
Title: RECOMBINANT STREPTOKINASE

REQUEST FOR GRANTING OF REFUND IMPROPER CHARGE TO DEPOSIT ACCOUNT

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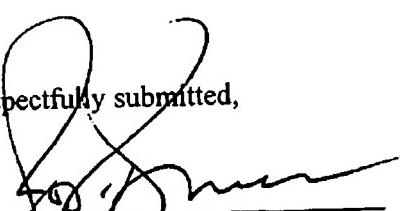
To the Commissioner:

On October 13, 2005, I submitted, on behalf of Applicants, a Response to the Office Action of June 13, 2005 and included a Petition for Extension of Time including the appropriate small entity one-month extension fee of \$60 by check number 80077. Copies of the Response and Petition for Extension of Time, each bearing a certificate of mailing dated October 13, 2005, are enclosed.

On November 3, 2005, our firm's Deposit Account No. 18-2055 was charged \$165 for a two-month extension fee (\$225 - \$60 paid by check). A copy of the deposit account statement containing the charge is enclosed.

Because the Response was filed within the one-month extension period, Applicants request a refund of \$165 by crediting Deposit Account No. 18-2055.

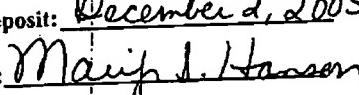
Respectfully submitted,


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IN THE UNITED STATES PATENT AND TRADEMARK Office

Appln. Serial No.: 09/882,509

Group Art Unit: 1652

Filing Date: June 15, 2001

Examiner: Monshipouri, M.

Applicant(s): KUPPUSAMY et al.

Attorney Docket No. 51321.003

Title: RECOMBINANT STREPTOKINASE

RESPONSE TO OFFICE ACTION, 37 CFR §1.111

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To the Commissioner:

In response to the Office Action dated June 13, 2005, the time period for response set to expire October 13, 2005, by virtue of the Petition for One-Month Extension of Time filed herewith, Applicants request favorable reconsideration in view of the following amendment and accompanying remarks.

Amendments to the Claims begins on page 2 of this paper.

Applicants' Remarks begin on page 5 of this paper.

IN THE CLAIMS

1. (CURRENTLY AMENDED) A DNA expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a DNA sequence encoding streptokinase, wherein the streptokinase has an amino acid sequence as encoded in the DNA sequence of SEQ. ID. NO. 3, and wherein the expression construct drives formation of inclusion bodies comprising enzymatically-active streptokinase in a host cell transformed to contain the expression construct, and wherein the streptokinase is enzymatically active upon solubilization of the inclusion bodies.
2. (ORIGINAL) The DNA expression construct of Claim 1, wherein the promoter is a λ pR- λ pL promoter.
3. (ORIGINAL) The DNA expression construct according to Claim 1, wherein the DNA sequence encoding streptokinase has a DNA sequence of SEQ. ID. NO. 3.
4. (CURRENTLY AMENDED) A method of producing streptokinase comprising transforming a host cell with an expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a DNA sequence encoding streptokinase, wherein the streptokinase has an amino acid sequence as encoded in the DNA sequence of SEQ. ID. NO. 3, and wherein the expression construct drives formation of inclusion bodies comprising enzymatically-active streptokinase in a host cell transformed to contain the expression construct; and then heat inducing the host cell, whereby wherein the host cell expresses inclusion bodies comprising

enzymatically-active streptokinase, and further wherein the streptokinase
is enzymatically active upon solubilization of the inclusion bodies.

5. (ORIGINAL) The method of claim 4, wherein the host cell is an *E. coli* cell.
- 6-7. (CANCELED).
8. (ORIGINAL) The method according to Claim 4, further comprising:
inoculating culture media with the transformed host; and
fermenting the transformed host.
9. (CURRENTLY AMENDED) The method according to Claim 8, further comprising isolating the enzymatically-active streptokinase produced.
10. (ORIGINAL) The method according to Claim 9, wherein the enzymatically-active streptokinase is isolated by steps comprising:
 - (a) pelleting the transformed host;
 - (b) disrupting the transformed host to release the inclusion bodies and partitioning the released inclusion bodies;
 - (c) isolating the partitioned inclusion bodies;
 - (d) solubilizing the isolated inclusion bodies;
 - (e) diafiltering the solubilized inclusion bodies;
 - (f) purifying the diafiltered inclusion bodies by ion exchange chromatography and then by gel permeation chromatography to separate fractions containing the streptokinase; and
 - (g) diafiltering the fractions containing the streptokinase.

11. (CURRENTLY AMENDED) A genetically-engineered host cell which expresses enzymatically-active streptokinase comprising: a host cell transformed to contain an expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a DNA sequence encoding streptokinase, wherein the streptokinase has an amino acid sequence as encoded by the DNA sequence of SEQ. ID. NO. 3, wherein the expression construct drives formation of inclusion bodies comprising enzymatically-active streptokinase in the host cell, and wherein the streptokinase is enzymatically active upon solubilization of the inclusion bodies.

12-21. (CANCELED).

REMARKS

Claims 1, 4, 9, and 11 have been amended herein. Support for the claims as amended can be found in the specification at page 3, third full paragraph; at page 4, first full paragraph; and at page 12, first and second full paragraphs. No new matter is added.

Claims 6, 7, and 12-21 were canceled in a previous paper.

Claims 1-5 and 8-11 remain in the application. Favorable reconsideration is respectfully requested.

Rejection of Claims 1-5, and 8-11 Under §112, Second Paragraph:

This rejection is believed to have been overcome by appropriate amendment to the claims. Specifically, the claims have been amended to recite that the streptokinase is expressed in the form of an inclusion body, and that the insoluble streptokinase contained in the inclusion bodies is enzymatically active upon solubilization of the inclusion body. This change in the positive recitation of the claims is believed to address the Examiner's concerns regarding using the adjective "enzymatically active" as applied directly to the inclusion body itself.

Applicants' intent with respect to the prior language of the claims was to require in the claims that the streptokinase expressed as an insoluble inclusion body is, in fact, "enzymatically active" when resolubilized, rather than being denatured and thus irreversibly inactivated. The claim language as amended is believed to clarify this point and thus overcome the rejection under §112, second paragraph. Withdrawal of the rejection is therefore respectfully requested.

Applicants wish to repeat here a point raised earlier: enzymes expressed in inclusion bodies are usually rendered irreversibly inactive because the enzyme becomes denatured in the inclusion body. See Applicants' prior response filed February 15, 2005 (in the paragraph spanning pages 7 and 8, and Exhibit A). In

short, one of ordinary skill in the art cannot and will not assume that an enzyme expressed as an inclusion body will be enzymatically active once the inclusion body is put back in solution. This is because most enzymes, when expressed as an inclusion body, are irreversibly denatured and thus enzymatically inactive, even when resolubilized. See Exhibit A of Applicants' response filed February 15, 2005.

In contrast, in the present invention the streptokinase is expressed in an inclusion body. That makes the enzyme far easier to isolate and purify. When the inclusion body is then resolubilized, the streptokinase is enzymatically active. Thus, the inclusion bodies themselves contain non-denatured, "enzymatically active" streptokinase.

Rejection of Claims 1, 3 and 11 Under §102(b) in View of Pupo et al. (1999)

Biotechnology Letters 21:1119-1123

The rejection of Claims 1, 3 and 11 is respectfully traversed because Pupo et al. clearly do not describe an expression construct which drives the expression of inclusion bodies comprising streptokinase (as required by Claims 1 and 3), nor do Pupo et al. describe a host transformed to contain such an expression construct (as required by Claim 11). Moreover, Pupo et al.'s insoluble fraction cannot have any endogenous streptokinase activity because, as per the Office's own definition, when an enzyme is insoluble it is inherently devoid of activity.

The first point, that Pupo et al. do not describe expressing streptokinase in an inclusion body, is made quite clear by the passage at page 1120, left-hand column, first full paragraph of Pupo et al.:

In this work we describe how the skc gene from *S. equisimilis* H46A can be expressed in *E. coli* W3110 as a soluble protein.... It was then possible to develop a simple procedure for the purification of the recombinant SKC while not including any solubilization or refolding of this protein. (Emphasis added.)

Pupo et al. express an enzymatically-active streptokinase enzyme in *E. coli* without any solubilization of the resulting enzyme. By the Office's own definition, the enzyme produced by Pupo et al. must already be in solution. (See page 4 of the Office Action, "When an enzyme is insoluble it is inherently devoid of activity.") If Pupo's enzyme was insoluble, it would not have any activity and a solubilization step would be required. Therefore, Pupo et al. does not disclose expressing streptokinase in an inclusion body because Pupo's method does not include any solubilization step. (See the above-quoted passage.) In short, If Pupo's streptokinase was expressed as an inclusion body (which is required by the positive language of the present claims), Pupo's method would have to include a solubilization step. But Pupo et al. explicitly state that their method does not include a solubilization step.

The Office expresses puzzlement that Applicants keep raising the fact that any streptokinase activity found in Pupo's insoluble fraction is simply contamination from Pupo's soluble fraction. The point is this: Pupo's insoluble fraction does not contain any streptokinase activity of its own. Pupo's streptokinase activity is found entirely in the soluble fraction. The only reason there is any streptokinase activity in Pupo's insoluble fraction is due to contamination from the soluble fraction. Pupo himself says so at page 1122, left-hand column, first paragraph.

This issue is directly at the heart of the rejection: Pupo et al. explicitly state that their construct drives the expression of streptokinase "while not including any solubilization step." (Pupo et al., page 1120, left-hand column, first full paragraph.) By definition, then, the streptokinase formed in Pupo's approach is not in the form of an inclusion body because an inclusion body is insoluble. Pupo's insoluble fraction does not contain any endogenous streptokinase activity. As noted by Pupo himself, the presence of a small amount of streptokinase in his insoluble

fraction was contamination from the soluble fraction. The Office itself points out that when an enzyme is insoluble it is inherently devoid of activity. The point is that Pupo et al. do not express streptokinase in the form of an inclusion body.

The Office has taken an exactly opposite position, a position that is flatly contradicted both by the passage from Pupo et al. at page 1122, left-hand column, first paragraph, and the Office's own statement that an insoluble enzyme is inherently devoid of activity. Specifically, the Office states, at the top of page 4 of the Office Action that:

Pupo does not need to explicitly say that the "insoluble fraction" is insoluble SKC. Any one of ordinary skill in the art is fully aware that once one prepares a recombinant enzyme (in this case being SKC) in inclusion bodies most (if not all) of said enzyme is in insoluble form.

But Pupo et al. do not teach expressing streptokinase in an inclusion body. As noted above, Pupo et al. describe a method of making streptokinase without any solubilization step. As a consequence, Pupo's insoluble fraction does not contain any endogenous streptokinase. As Pupo himself stated, the small amount of streptokinase activity found in the insoluble fraction was contamination from the soluble fraction. Pupo et al., page 1122, left-hand column, first paragraph. The entire aim and goal of Pupo et al.'s work, as is clearly stated at page 1120, left-hand column, first full paragraph, was to make "a soluble protein.... while not including any solubilization... of the protein." In short, Pupo et al. do not describe constructs that drive the expression of inclusion bodies that comprise streptokinase.

The Office Action also states, at the bottom of page 4 of the Office Action, that "Pupo is merely cited to indicate that SKC preparation in inclusion bodies has been done prior to this invention." In reply, Applicants note that Pupo et al. do not teach the expression of streptokinase in inclusion bodies. Moreover, the present claims are not directed solely to the expression of streptokinase in inclusion bodies. The present claims positively require a construct that drives the expression of

streptokinase in the form of an inclusion body and further that the streptokinase be enzymatically active when the protein is solubilized. This claimed invention simply is not taught, nor is it suggested, by Pupo et al. themselves nor any of the references cited by Pupo et al.

The Office also states, in the sentence spanning pages 4 and 5 of the Office Action, that "The method used to remove contamination from [the insoluble] SKC and what Pupo should have done to avoid contamination are totally irrelevant."

Applicant agree entirely, but that was not the Applicants' prior argument.

Applicants' earlier-submitted and current argument, which is critically relevant, is that Pupo's method drives the expression of soluble streptokinase in the absence of a solubilization step. The only conclusion that can be drawn from Pupo's exact words are that his insoluble fraction is devoid of endogenous streptokinase activity. In contrast, the present claims require that an enzymatically active streptokinase be expressed in the form of an insoluble inclusion body.

Specifically regarding Claim 3, this claim requires the exact DNA sequence shown in Applicants' SEQ. ID. NO: 3. Pupo et al. simply do not disclose the required DNA sequence. Therefore, the Pupo et al. paper cannot anticipate Claim

3.

Because Pupo's insoluble fraction is devoid of any endogenous streptokinase activity, while all of the present claims are directed to constructs or methods that express streptokinase in the form of an insoluble inclusion body, Applicants submit that the rejection of Claims 1, 3, and 11 under §102(b) in view of Pupo et al. is untenable. Withdrawal of the rejection is respectfully requested.

**Rejection of Claims 2, 4-5 and 8-10 Under §103(a) over Pupo et al. (1999)
Biotechnology Letters 21:1119-1123 in view of U.S. Patent No. 5,708,148 to
Schmitz et al.**

This rejection is respectfully traversed because recombinant IGF-II, as taught in Schmitz et al., is a totally different protein than streptokinase. IGF-II is totally unrelated to streptokinase, has a totally different amino acid sequence than streptokinase, and has no logical link to streptokinase in any fashion. In short, a person of ordinary skill would not conclude, nor would be provided any reasonable expectation of success, that simply because IGF-II can be solubilized from an inclusion body, so too could streptokinase. The production of recombinant, enzymatically active enzymes simply cannot be generalized in this fashion.

The remarks provided above with respect to the Pupo et al. paper are repeated herein in full. Briefly recapping, Pupo et al. do not describe an expression construct which drives the expression of inclusion bodies comprising streptokinase, nor do Pupo et al. describe a host transformed to contain such an expression construct. And Pupo et al.'s insoluble fraction cannot have any endogenous streptokinase activity because, as per the Office's own definition, when an enzyme is insoluble it is inherently devoid of activity.

Streptokinase is a single chain polypeptide having 415 amino acid residues. See page 2, first full paragraph of the specification as filed and SEQ. ID. NO: 3. In stark contrast, IGF-II is only 67 amino acids long. See Schmitz et al., column 1, line 55, and SEQ. ID. NO: 1 of Schmitz et al. In short, the construct claimed by Applicants drives the expression of an inclusion body comprising streptokinase, a protein that is 519% larger than the IGF-II described in Schmitz.

Applicants therefore submit that this rejection is clearly improper. There is no reasonable likelihood of success, based on Schmitz' ability to express IGF-II in an inclusion body and to recover the active IGF-II protein, that the same can be done with streptokinase, a protein that is more than 500% larger than IGF-II.

Proteins simply cannot be generalized in this fashion. The IGR-II protein described in Schmitz is vastly smaller than the streptokinase recited in Applicants' claims. The IGF-II protein is thus easier to solubilize than is streptokinase due to its vastly smaller molecular weight as compared to the molecular weight of streptokinase.

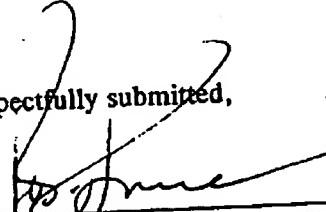
It is well known that proteins can and do have vastly different qualities, from egg whites to human hair. The simple fact that Schmitz can manipulate IGF-II into an inclusion body, and then solubilize the inclusion body to yield active IGF-II does not mean that the same can be done with streptokinase.

Applicants therefore respectfully submit that the rejection of Claims 2, 4-5 and 8-10 under 35 USC §103(a) is untenable. Withdrawal of the same is respectfully requested.

CONCLUSION

Applicants submit that the application is now in condition for allowance. Early notification of such action is earnestly requested.

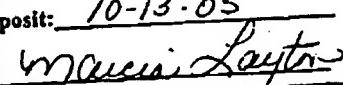
Respectfully submitted,


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Appln. Serial No.: 09/882,509

Group Art Unit: 1652

Filing Date: June 15, 2001

Examiner: Monshipouri, Maryam

Applicants: Kuppusamy et al.

Attorney Docket No.: 51321.003

Title: RECOMBINANT STREPTOKINASE

PETITION FOR EXTENSION OF TIME UNDER 37 C.F.R. §1.136(a)

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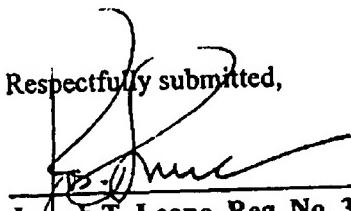
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To the Commissioner:

Applicants petition the Commissioner of Patents and Trademarks to extend the time for response to the Office Action dated June 13, 2005 for one month from September 13, 2005 to October 13, 2005. The granting of the requested extension of time will not extend the period for response beyond the six-month statutory limit.

A check for \$60 is enclosed to cover the petition fee. The Commissioner is hereby authorized to charge any additional fees or credit any overpayment to Deposit Account No. 18-2055.

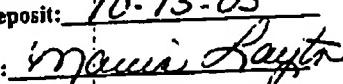
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